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ATOMIC FORCE MICROSCOPY OF DNA IN AQUEOUS SOLUTIONS

by

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DNA on mica can be imaged in the atomic force microscope (AFM) in water or in some buffers if the sample has first been dehydrated thoroughly with propanol or by baking in vacuum and if the sample is imaged with a tip that has been deposited in the scanning electron microscope (SEM). Without adequate dehydration or with an unmodified tip, the DNA is scraped off the substrate by AFM-imaging in aqueous solutions. The measured heights and widths of DNA are larger in aqueous solutions than in propanol. The measured lengths of DNA molecules are the same in propanol and in aqueous solutions and correspond to the base spacing for B-DNA, the hydrated form of DNA; when the DNA is again imaged in propanol after buffer, however, it shortens to the length expected for dehydrated A-DNA. Other results include the imaging of E.coli RNA polymerase bound to DNA in a propanolwater mixture and the observation that washing samples in the AFM is an effective way of disaggregating salt-DNA complexes. The ability to image DNA in aqueous solutions has potential applications for observing processes involving DNA in the AFM.

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Atomic force microscopy of DNA in aqueous solutions

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ABSTRACT

DNA on mica can be imaged in the atomic force microscope (AFM) in water or in some buffers if the sample has first been dehydrated thoroughly with propanol or by baking in vacuum and if the sample is imaged with a tip that has been deposited in the scanning electron microscope (SEM). Without adequate dehydration or with an unmodified tip, the DNA is scraped off the substrate by AFM-imaging in aqueous solutions. The measured heights and widths of DNA are larger in aqueous solutions than in propanol. The measured lengths of DNA molecules are the same in propanol and in aqueous solutions and correspond to the base spacing for B-DNA, the hydrated form of DNA; when the DNA is again imaged in propanol after buffer, however, it shortens to the length expected for dehydrated A-DNA. Other results include the imaging of *E.coli* RNA polymerase bound to DNA in a propanol-water mixture and the observation that washing samples in the AFM is an effective way of disaggregating salt-DNA complexes. The ability to image DNA in aqueous solutions has potential applications for observing processes involving DNA in the AFM.

INTRODUCTION

Biological processes take place in aqueous environments. The atomic force microscope (AFM)[1,2] can image molecules in aqueous or other fluid environments by scanning a tiny tip over a surface to which the molecules are bound. Thus it is reasonable to expect that the AFM will be able to image biomolecular processes as they are occurring. This expectation was first fulfilled several years ago with the filming of fibrin polymerizing in the AFM.[3] Extension of such work to DNA has been hampered by the difficulty of obtaining stable reproducible images of DNA in aqueous solution in the AFM. Lyubchenko et. al.[4] have succeeded in imaging long strands of DNA bound to silylated mica under water. In this work the entire 17-μ lambda phage genome has been imaged in a single scan but with apparent DNA widths of tens of nanometers. The present work presents atomic force microscopy of smaller plasmid DNAs under water and HEPES buffer with a resolution of several nanometers. This has potential applications to molecular-resolution imaging of processes involving DNA.

METHODS

Materials

Ruby mica was obtained from New York Mica Co., New York, NY and was freshly cleaved before use. Bluescript II SK M13(+) double-stranded plasmid DNA (2960 base pairs, 1 mg/ml) and lambda/HindIII DNA markers (250 μg/ml) were obtained from Stratagene, LaJolla, CA, supplied in 10 mM Tris, 1 mM EDTA.

Sample preparation

Bluescript and lambda/HindIII DNA samples were diluted with water and were prepared on mica in one of three ways (see figure captions): (1)on mica pretreated with magnesium acetate, as described previously,[5–9] (2) on mica pretreated with 10 mM calcium acetate, following the same procedure otherwise, or (3) on untreated fresh-split mica. Samples typically contained 50 ng DNA and were dried in vacuum over Drierite for 15 minutes or more before AFM-imaging.

pUC9 DNA and a plasmid isolated from a derivative of *E.coli* strain HB101(HB101 plasmid) were suspended at a concentration of 2 mg/ml in 30 mM triethanolamine-HCl (pH 7.9, 10 mM $MgCl_2$, 0.1% glutaraldehyde) as described earlier.[10] This was adsorbed onto a freshly cleaved mica surface (Marivac Ltd., Halifax) and allowed to air dry. It was then washed in bidistilled water and ethanol prior to storage until use. To form transcription complexes of *E.coli* RNA polymerases bound to supercoiled pUC9 plasmid DNA (2673 bp), the specimens were prepared according to a method described by ten Heggeler-Bordier et al. [11] and Klaus et al. [12] These samples were also observed by conventional electron microscopy as previously described.[10,13]

AFM-imaging

Atomic force microscopy was done under propanol or aqueous solutions using a Nanoscope II AFM (Digital Instruments, Santa Barbara, CA) as described previously.[9] Silicon nitride cantilevers with integrated tips were supplied by Digital Instruments (NanoProbes and a prototype of improved NanoProbes) and Olympus (Olympus Opt. Co. Ltd., Tokyo); supertips[8,14] were deposited onto the NanoProbes and improved

NanoProbes in the scanning electron microscope (SEM) as noted in the figure captions. New cantilevers were generally used for each experiment. Images were taken without on-line filtering and were subsequently processed only by flattening to remove the background slope. Information density of captured images was 400 pixels per line for 400 lines.

Statistics

Statistical significance was determined with the Wilcoxon Signed-Rank Test for paired samples and the Wilcoxon Rank-Sum Test for independent samples.[25] Paired samples were the heights and widths in propanol and aqueous solutions measured with the same tip. Independent samples were the base spacing of Bluescript, which was calculated from the measured lengths.

RESULTS

Plasmid DNA can be imaged in aqueous solution if it has been pretreated with propanol and if it is imaged with a tip deposited in the SEM

When DNA is imaged in water without propanol pre-treatment, the DNA is rapidly scraped off the mica. If the DNA is first imaged in propanol, however, it can be subsequently imaged in some aqueous environments with an SEM-deposited tip. Stable images have been obtained in water (Fig. 1; Fig. 3b and c), water-propanol mixtures (Fig. 3d), and HEPES buffers containing 2 to 10 mM HEPES pH 7.6 with or without 1 mM $MgCl_2$ (Fig. 2a–e; Fig. 3a). Imaging in HEPES buffers is most successful after imaging in water. The addition of 25 mM NaCl

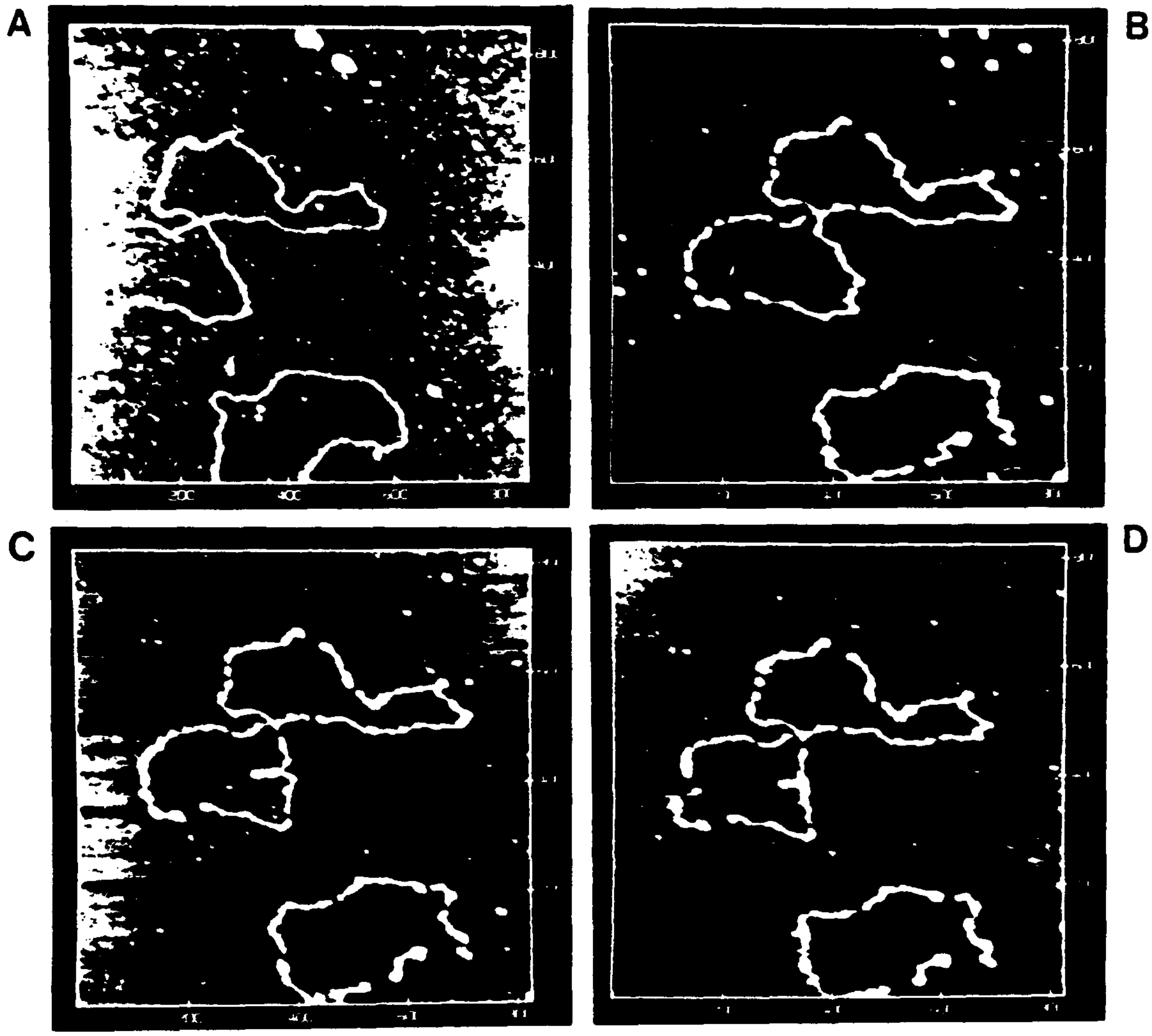

Figure 1. Atomic force microscopy of the same plasmid DNA molecules in propanol and water showing the stability of imaging in water. HB101 plasmid DNA in glutaraldehyde, triethanolamine and $MgCl_2$ was deposited onto fresh-split mica. Sample was imaged with a NanoProbe 100-μ narrow cantilever with a 5-second tip deposited in the SEM. Scan speed, 8.7 Hz. 820-nm images are taken from scans of 820 to 850 nm. (A) DNA in propanol. The DNA was imaged in propanol for 45 minutes before imaging in water. (B) The same DNA molecules after 24 minutes of continuous scanning in water. (C) After 33 minutes in water, the middle plasmid has developed a fold on the right side. (D) The final image, after 1 hour of imaging in water, shows that the DNA is still bound to the mica and retains approximately its original shape.

destabilizes the binding of DNA to mica so much that the DNA is damaged even with scan sizes as large as 2μ. Even under optimum imaging conditions, DNA is more easily damaged or moved in aqueous solutions than it is in propanol (cf. Fig. 1a and b). This observation seems reasonable, since DNA is insoluble in propanol but is soluble in water, where it is probably loosened from the mica in some places, giving rise to loops which are then susceptible to being cut or pushed.

More recent results show that DNA can be imaged directly in water or HEPES buffer if it has been thoroughly dehydrated by baking in vacuum. This is reasonable, since propanol also dehydrates DNA effectively. Best results were obtained when the DNA on mica was placed on a hot metal block at 100°C in vacuum in a desiccator for 3 hrs or more. Vacuum treatment alone does not dehydrate the DNA enough for aqueous imaging.

Other factors also contribute to success in aqueous imaging. Slow scan rates and large scan sizes are generally less destructive to the DNA. Fig. 2b shows that at a scan rate of 8.7 Hz the DNA is being damaged, while at 5.8 Hz (Fig. 2c) the DNA shows little further damage even though it has been imaged continuously for 7 minutes at this scan rate. These images are taken from 195-nm scans, which are smaller areas than can usually be imaged in aqueous solutions. Imaging at slow scan rates is not always trivial, however, since there is often a significant drift resulting in increased imaging forces, which tend to scrape the DNA away, or decreased imaging forces, in which case the tip lifts off the sample.

DNA can be stable in water for a long time. The DNA in Fig. 1 was imaged continuously for 1 hour, and the DNA in Fig. 3c was imaged for several minutes after being in water for 3 hours.

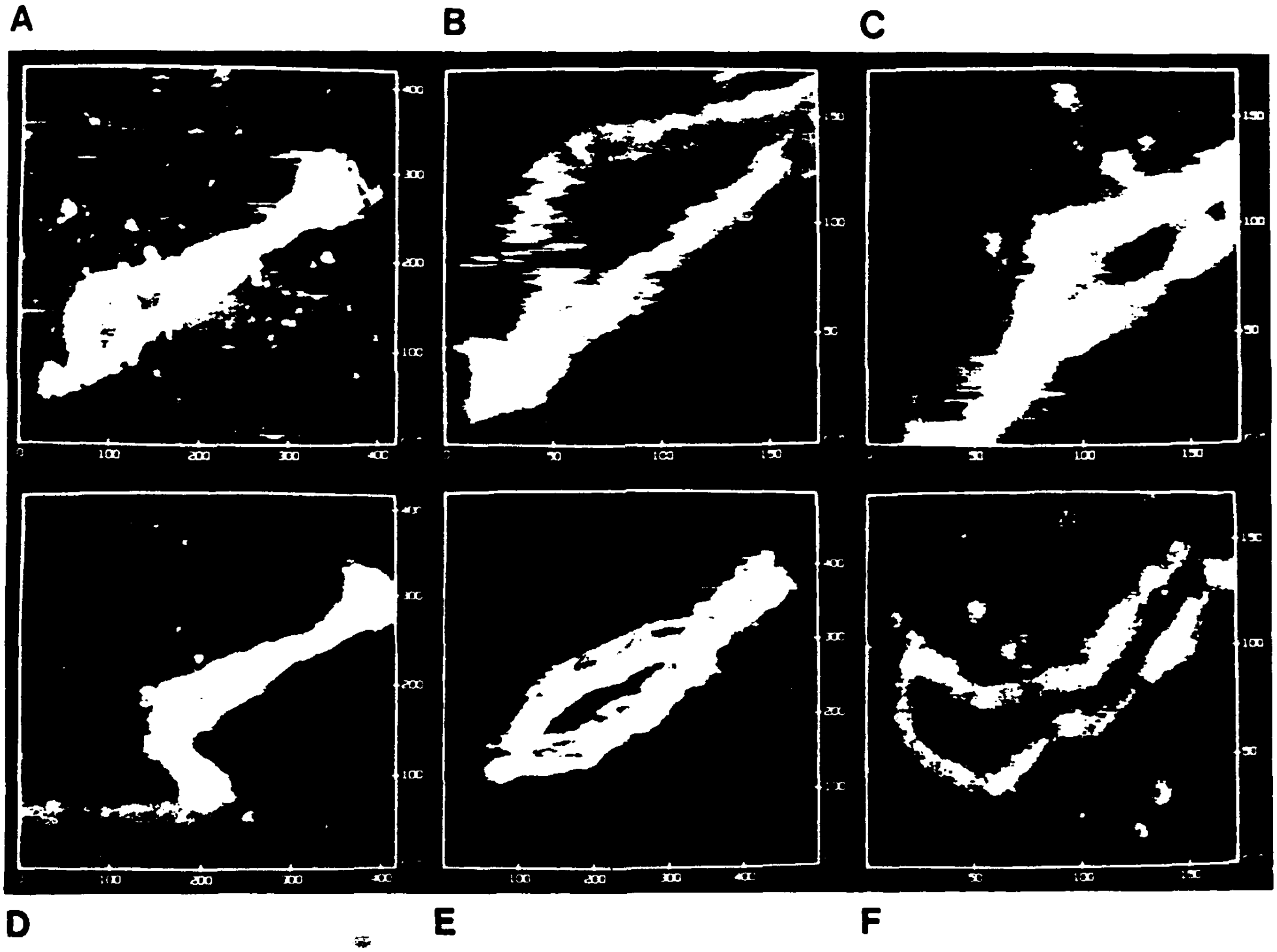

Figure 2. Atomic force microscopy of Bluescript plasmid DNA after prolonged imaging in HEPES buffers (A to E) and propanol (F). DNA on Mg-treated mica was imaged with a NanoProbe 200-μ wide cantilever with a 1-minute tip deposited in the SEM. DNA was imaged 15 minutes in propanol, followed by 40 minutes in water, 70 minutes in 10 mM HEPES, pH 7.6, 20 minutes in HEPES buffer with 1 mM $MgCl_2$, and finally again in propanol. (A) to (D): Images of the same plasmid in HEPES, with an apparent width of 20 nm and evidence of a double tip. Whole plasmid (A), followed by smaller scans (B and C); subsequent imaging of whole plasmid (D) shows that parts of plasmid have been moved by scanning. (E) A different plasmid in HEPES + $MgCl_2$ showed no damage or change in shape after 3 1/2 minutes of continuous scanning. (F) Width in propanol after aqueous imaging is 7–10 nm, with evidence of a double tip. Scan speeds 8.7 Hz except for (C), 5.8 Hz. Image sizes, 416 nm (A and D), 172 nm (B, C, and F) and 500 nm (E). Original scan sizes, 1000 nm (A), 200 to 240 nm (B, C and F), 500 to 570 nm (D and E).

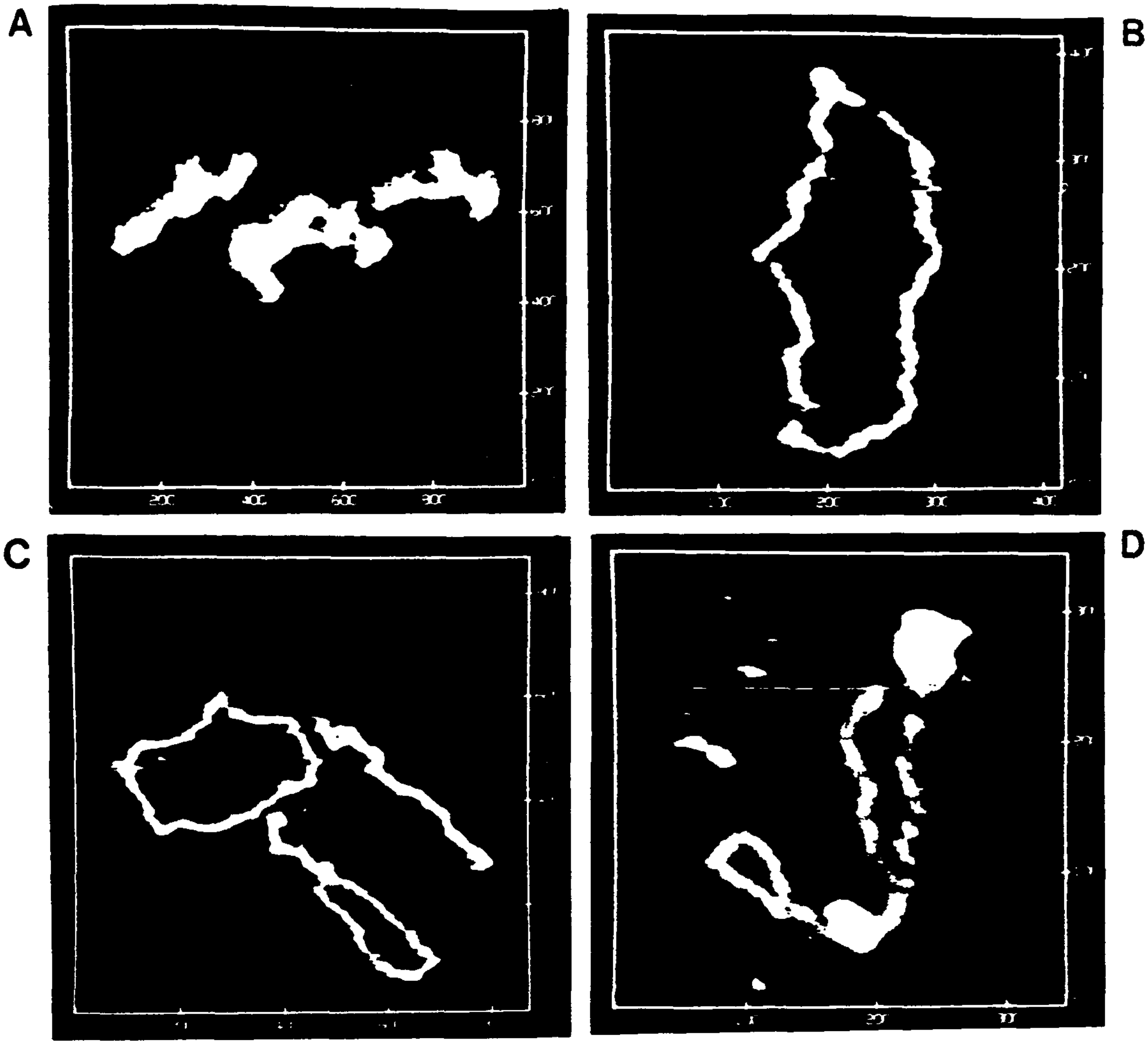

Figure 3. Plasmids imaged in aqueous solutions from several different experiments. (A) A cluster of Bluescript plasmids in 5 mM HEPES after 72 minutes in water and in HEPES. Plasmids on fresh-split mica were imaged at 8.7 Hz with a 5-second SEM-deposited tip on a 100-μ narrow NanoProbe cantilever. The same plasmid cluster was imaged in water and HEPES over a 1-hour period with little change in conformation. Image and scan sizes 920×920 nm. (B) Bluescript plasmid after 40 minutes in water on fresh-split mica imaged at 7.1 Hz with a 1-minute SEM-deposited tip on a 200-μ wide cantilever with improved NanoProbe. This plasmid shows some damage after 12 minutes of continuous imaging. Another plasmid in the same experiment, which had its long axis oriented in a horizontal direction, i.e., in the direction of the scanning, showed no damage under similar conditions. (C) Bluescript plasmids after 3 hours in water on calcium-treated mica imaged with a 100-μ narrow NanoProbe cantilever with a brief deposition in the SEM. 860-nm image from a 980-nm scan after 5 minutes of continuous scanning of these plasmids. A vertically oriented plasmid from the same experiment was more easily damaged. There is also evidence from other experiments, such as (A), that horizontal DNA strands are more resistant to damage in the AFM than vertical DNA strands. (D) Plasmid pUC9 with RNA polymerase imaged in a mixture of 40% water−60% iso-propanol with a 100-μ narrow NanoProbe cantilever with an SEM-deposited tip.

Another factor that may contribute to stable imaging is the use of glutaraldehyde in sample preparation, as in Fig. 1 and 4d; this has not been investigated thoroughly. Glutaraldehyde would make the DNA less flexible by forming Schiff bases with the amino groups on adjacent DNA bases. A disadvantage of using glutaraldehyde, and anything that is not volatile, is that much more washing of the samples is necessary.

The other requirement for successful aqueous imaging is an SEM-deposited tip. DNA in aqueous solutions is always scraped away with unmodified tips. Such tips can be converted to tips suitable for imaging DNA in aqueous solution by even a very brief SEM-deposition. The tips used for Figs. 1 and 3a, for example, were grown with only a 5-second deposition. It appears that the important difference between the unmodified silicon nitride tips and the SEM-deposited carbon tips is in their surface chemistry. The surface of silicon nitride tips is glass-like: i.e., it is covered with Si-OH groups,[16] while carbon tips are more non-polar.

For imaging in propanol alone, improved NanoProbes and Olympus tips were preferred because they gave more reliably narrow apparent widths of DNA than SEM-deposited tips or standard NanoProbes. SEM-deposited tips are highly variable; some of the narrowest DNA images have been obtained with these tips, but many of the tips are multiple. There is no good way to determine which tips will give multiple-tip images of DNA except by imaging DNA.

Force curves (Fig. 4) are an indication of the nature of the interaction between the tip and the substrate. The force curves

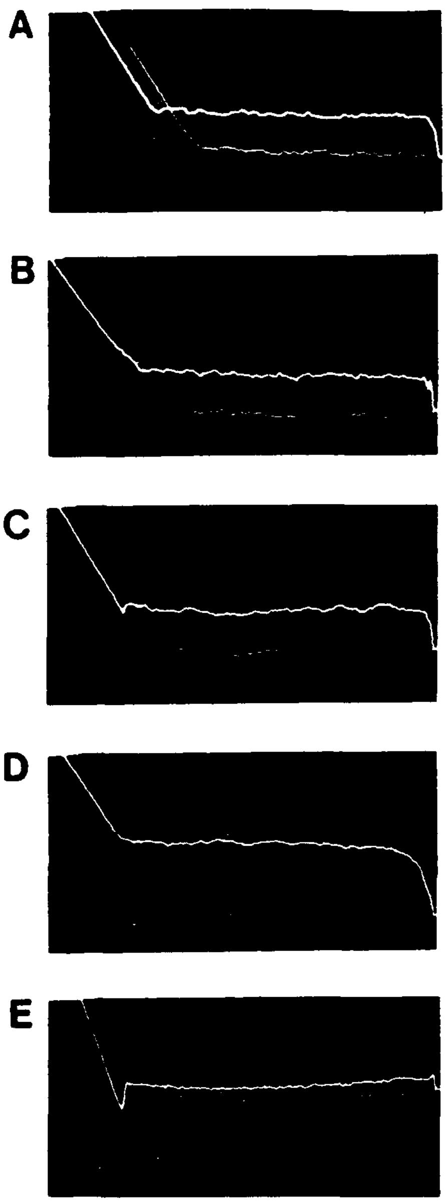

Figure 4. (A) to (D) are force curves from the same experiment as the images in Fig. 2 in (A) water, (B) 10 mM HEPES, pH 7.6, (C) HEPES + 1 mM $MgCl_2$, and (D) propanol. Curve D was recorded after the aqueous solutions of A to C. Note the clean appearance of the curves and the slight repulsion seen in HEPES without $MgCl_2$ (C; see results). In generating force curves the AFM tip moves vertically up and down above the sample, alternately approaching the sample (upper trace) and withdrawing from it (lower trace). Y-axis measures the cantilever deflection; X-axis measures the position of the sample; the curve bends upward when the tip touches the sample. (E) When the tip sticks to the substrate, there is a downward spike in the lower trace as the tips begins to lift off the sample.[8] This force curve was taken immediately after engaging the tip to a sample in water.

in Fig. 4a to d were recorded during the course of the experiment illustrated in Fig. 2. They show no adhesive component; i.e., the tip was not sticking to the substrate, which may also have contributed to the stability of the images in Fig. 2. The force curve in HEPES (Fig. 4b) shows a slight repulsion between the tip and the substrate, visible in the slight curvature of the slanted portion of the force curve and the slight upward slope of the horizontal portion. This is typical for force curves in HEPES on mica; the repulsion can be eliminated by adding $MgCl_2$ to the buffer (Fig. 4c). It is useful to monitor the force curve periodically while imaging DNA. There are two reasons for this: first, to determine whether the cantilever has drifted to high force and, if so, to lower the force, and second, to determine whether the tip is sticky, which can be seen by the presence of a variable adhesive component in the force curve (e.g., Fig. 4e). Sometimes the force curve will show an adhesive component immediately *after engaging* the tip to the sample (Fig. 4e); this adhesive component often disappears after a few minutes of imaging.

When imaging DNA in propanol, it is usually possible to obtain stable images of 50-nm scan sizes or less, even with an unmodified tip. When imaging in aqueous solution, it is usually necessary to have scan sizes of 500 nm or more to obtain stable images, although occasionally stable images have been obtained with 200 nm scans, as in Fig. 2c and d. Thus, imaging in aqueous solutions is useful primarily for applications in which resolution at the level of entire plasmids is sufficient.

Table 1. Bluescript DNA in propanol and aqueous solutions[a]

	Measured widths (nm)	Measured heights (nm)	Bluescript base spacing (Å/bp)
In propanol before aqueous solution:	$9 \pm 3^{b,e}$	1.6 ± 0.5^{c}	3.4 ± 0.3^{d}
In aqueous solution	19 ± 4^{b}	2.5 ± 0.5^{c}	3.3 ± 0.3
In propanol after aqueous solution:	12 ± 4^{e}	1.6 ± 0.2	2.8 ± 0.3^{d}

[a] Means ± S.D. Measured heights and widths are from 6 to 12 separate experiments in each group. Base spacing is calculated from measured lengths of 12 to 19 plasmids in each group.
[b] Statistically significant difference ($p > 0.01$) between measured widths in aqueous solution and in propanol before aqueous solution.
[c] Statistically significant difference ($p > 0.01$) between measured heights in aqueous solution and in propanol before aqueous solution.
[d] Statistically significant difference ($p > 0.01$) between calculated base spacing in propanol before and after buffer.
[e] Difference not statistically significant (N.S.) between measured widths in propanol before and after aqueous solution.

DNA in aqueous environments appears to be higher and wider than DNA in propanol

Measured heights and widths of DNA are highly variable, even within a single molecule, since the DNA strands often look somewhat like a string of beads in the AFM. Nonetheless there is a significant increase ($p < 0.01$) in the apparent heights and widths of DNA in aqueous solutions (Table 1). This is evident also from a comparison of images in propanol, e.g., Figs. 1a and 2f, with corresponding images in aqueous solutions (e.g., Figs. 1b and 2c). The increases in height and width suggest that the AFM tip is imaging a hydration layer around the DNA in water, although other explanations are possible. For example, there may be a greater electrostatic repulsion between the DNA and the tip in aqueous environments than in propanol. When DNA is again imaged in propanol after aqueous imaging, the heights and widths usually approach their original propanol values (Table 1).

The nucleotide spacing of Bluescript DNA in propanol, calculated from the measured plasmid lengths in propanol, is 3.4 ± 0.3 Å/base pair (bp). This is equal to the value for B-DNA, the hydrated form of DNA (3.4 Å/bp), instead of A-DNA, the

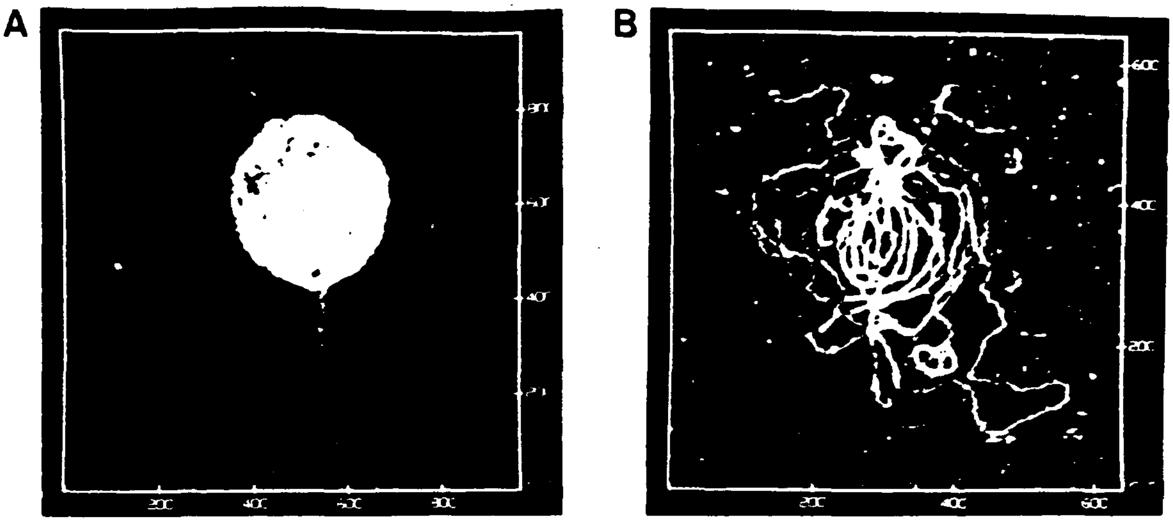

Figure 5. Lambda HindIII DNA on fresh-split mica imaged in propanol before (A) and after (B) a water-wash. Measured width of DNA is 8–10 nm (B). Images taken with improved NanoProbe at 7 Hz scan rate. (A) 960 nm image from 1260 nm scan; (B) 640 nm image from 840 nm scan.

dehydrated form (2.9 ± 0.4 Å/bp),[17] which would be expected in propanol. The DNA is applied to mica in aqueous solution, and the mica is dried before imaging in propanol. Thus it appears that the length of DNA is determined by the aqueous environment in which it is bound to mica and that vacuum-drying and propanol-treatment do not shorten the bound DNA molecules.

The measured lengths do not change when plasmids are imaged in water or buffer, but plasmids are often shorter, comparable in length to A-DNA, when imaged again in propanol after being buffer (Table 1). In Fig. 1, for example, the same molecules of DNA are imaged in propanol and in an aqueous environment without showing any significant change in either shape or length. When plasmids are imaged in propanol after exposure to buffer, they often appear to be more coiled as well as shortened. The cause of this shortening may be that some plasmids are partially loosened from the mica in buffer and, if not exposed to vacuum drying, are then precipitated in the A-form by propanol.

Complexes of DNA with RNA polymerase can be imaged in aqueous solution

E.coli RNA polymerase bound to plasmid pUC9 DNA was imaged in propanol and in 60% propanol-40% water (Fig. 3d). The lumps on the DNA strands measured 7 ± 2 nm high and 41 ± 13 nm wide (n = 14). When the same molecules were imaged in the two fluids, the dimensions of their lumps were similar in both fluids. Other regions of the same sample were imaged in the transmission electron microscope (TEM), which showed fields of molecules with an appearance characteristic of RNA polymerase bound to DNA. Zenhausern, et al.,[10] have previously reported that there is good agreement between AFM and TEM images of DNA and of DNA with RNA polymerase. *E.coli* RNA polymerase has a molecular weight of 430 kd[18] and is a highly asymmetric molecule.

Washing samples with water in the AFM can be used to remove bound salts

Some DNA samples such as the lambda/HindIII DNA in Fig. 5a show fields of usually circular masses of various sizes (Fig. 5a). These can often be dissociated with water into clusters of DNA strands. To do this, the propanol in the fluid cell of the AFM is replaced with water; after several minutes of soaking in water, the DNA is again imaged in propanol, giving fields of DNA clusters of various sizes, e.g., Fig. 5b. Similar, though less dramatic, results were reported earlier, in which plasmid networks were dissociated by washing the sample, outside the AFM, with warm water.[9] We assume that the circular masses of DNA were held together by salts, since the DNA polyanion would not aggregate in the absence of counter-ions. This sort of aggregation has been seen in phosphate buffer and in Tris-EDTA with and without NaCl, so it appears to be independent of any particular ion. Our current opinion is that aggregation can be minimized by using compressed air to blow the DNA solution off the mica and by minimizing the amount of DNA and the concentration of salts and buffers applied to the mica. The benefits of using compressed gas have also been reported by Shaiu et al.,[19] who have found that an orthogonal flow of nitrogen gas is helpful for orienting DNA attached to gold particles.

DISCUSSION

Aqueous imaging

The ability to image DNA in aqueous solutions is necessary for any observations of processes involving DNA, such as the interaction of DNA with enzymes. Two procedures for imaging DNA in water are now known: that of Lyubchenko, et al.,[4] and the procedure reported here. These methods differ in that Lyubchenko's method uses mica coated with an amino-silane and has been applied to 17-μ lambda DNA, while this method uses bare mica and has been applied to 1-μ plasmid DNA. Since we have not compared these two methods, only the method reported here will be discussed in detail.

Two essential prerequisites for the aqueous-imaging method presented here are: (1) a thorough dehydration of the DNA by baking in vacuum or pretreatment with propanol and (2) imaging with a tip deposited in the SEM. Dehydration probably allows the DNA to bind more tightly to the mica by removing water molecules between the DNA and the mica. The necessary property of SEM-deposited tips seems to be their surface chemistry, since silicon nitride tips that cannot image DNA in water can be converted into usable tips by SEM-deposition. The carbon tips grown in the SEM are assumed to be more non-polar than the silicon-nitride tips, which are glass-like on their outermost surface.[16] Preliminary results suggest that silicon nitride tips may be damaging and moving DNA by means of an electrostatic repulsion between the tip and the DNA. This repulsion seems to be absent from the interaction between DNA and SEM-grown carbon tips.

Two alternate explanations of the need for carbon tips are that the carbon tips are blunter and, hence, gentler than silicon nitride tips or that stiffer cantilevers are causing the DNA damage. Since the same cantilevers have been used with carbon tips and with silicon nitride tips, this does not seem to be the problem. We also have no evidence that DNA damage correlates with tip sharpness. The classical view is that pressure on the sample should increase as tips become sharper, due to the decrease in surface area of the tip. In practice, we have found that sharp tips are at least as gentle as blunt tips in imaging DNA. If electrostatic repulsion is responsible for much of the DNA damage, this could explain why sharp tips are perhaps even better than blunt tips. Hoh, et al.,[16] show that repulsion decreases at low pH for silicon nitride tips on a glass surface; the effect of lower pH has not been investigated for DNA in water or buffer on mica.

Although a slight repulsion may be responsible for much of the DNA damage seen with silicon nitride tips, it is also true that very adhesive, 'dirty' tips are likely to damage DNA. Such tips give force curves resembling that in Fig. 4e. The adhesion sometimes diminishes with time, resulting in a force curve more like the one in Fig. 4c. This adhesive 'dirt' on the tip may be simply DNA or DNA with salts and buffers, since DNA in salts and buffers seems to be quite attracted to itself, as seen from the aggregation in Fig. 5a.

DNA damage in both aqueous solutions and propanol is minimized by using large scan sizes or slow scan speeds. Slow speeds should allow the feedback system more time to respond to height changes, thus minimizing fluctuations in the imaging force, for a particular gain setting. Large scan sizes will increase the distance between successive scan lines, since each image is composed of 400 scan lines. It seems reasonable that DNA scanned every 5 nm, as in a 2000 nm image, will show less damage than DNA scanned every 0.5 nm, as in a 200 nm image.

Mica treatment

The mica treatment varied in these experiments from none (fresh-split mica) to pre-treatment with magnesium acetate or calcium acetate. Fresh-split mica is increasingly becoming the substrate of choice. There is no convincing evidence that pre-treatment with magnesium or calcium salts makes mica a better substrate for binding DNA. Furthermore, there is evidence that calcium and magnesium are readily rinsed off mica with water, especially when the mica is sonicated as described[8] (J.Israelachvili, personal communication).

DNA dimensions

Several interesting changes were observed in the apparent dimensions of DNA in aqueous solutions and in propanol. These changes are both intriguing in themselves and important in demonstrating the usefulness of the AFM for investigating the behavior of individual DNA molecules under different conditions.

The apparent heights and widths of DNA were 50–100% higher in water or buffer than in propanol. Since both the height and the width of the DNA increased, the tip must for some reason be traveling farther away from the DNA in aqueous solution. Possible explanations for this are that a hydration layer on the DNA prevents the close approach of the tip or that there is a repulsion between the tip and the DNA in aqueous solution. The influence of hydration on DNA dimensions might be investigated by imaging DNA in a series of alcohol-water mixtures, if the hydration of DNA is known as a function of alcohol concentration. If there is a repulsion between the tip and the DNA in aqueous solution, it is most likely to be electrostatic. Electrostatic effects might be studied by varying the pH of the solution. Varying the salt concentration would also affect the electrostatic repulsion, but salt removes the DNA from the mica surface.

For DNA in air, Vesenka, et al.,[20] propose that the dependence of height on humidity is due to the hydration of a salt layer over the mica surface in which the DNA is embedded. There is not much of a salt layer on samples of Bluescript plasmids on mica, since the Bluescript supplied by Stratagene has only 1.6 mg Tris + EDTA per mg DNA, as calculated from their concentrations.

DNA lengths show an interesting change from B-DNA to A-DNA. Unexpectedly, the measured lengths of DNA during the initial propanol imaging correspond to B-DNA, the hydrated form, apparently because the DNA is bound tightly to the mica from an aqueous solution. When imaged in propanol *after* imaging in buffer, however, the DNA has shortened to the dehydrated form, A-DNA. The explanation for this is probably that the DNA is loosened from the mica by buffer treatment, allowing it to dehydrate and shorten upon re-exposure to propanol. This is consistent with the observation that DNA is less stable in buffer than in propanol and is even removed from the mica altogether when salt is added to the buffer.

This work adds to a growing body of applications for atomic force microscopy of DNA. Guanine-rich nucleic acid structures[21] and complexes of RNA polymerase bound to DNA[5,10] have been imaged in air. Single-stranded DNA[9] and nucleosomes[6] have been imaged in propanol. The agreement between AFM and TEM results has been documented.[10,13] Measured heights of DNA in air have been shown to vary with the humidity[22] and the rotation of scan direction,[10] apparently because of friction between the cantilever and the sample. The height of DNA in propanol does not vary with rotation, probably because there is little friction in propanol, as seen from the force curve.[8] The AFM has been used to measure lengths of nucleic acid molecules[23] and to dissect them.[7,8,24] Atomic force microscopy of nucleic acids is rapidly improving with advances in AFM-design, tips, and sample preparation. Future goals include the imaging of processes involving DNA, higher resolution imaging of DNA-protein interactions, and perhaps even the sequencing of DNA.[25,26]

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